BULBILS OF LYCOPODIUM LUCIDULUM

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(WITH TWENTY-ONE FIGURES)

It is well known that certain species of *Lycopodium* belonging to that subdivision of the genus which is characterized by the non-strobilar arrangement and slight differentiation of the sporophylls produce organs of vegetative reproduction known as bulbils or gemmae. These organs are of a peculiar type, so unlike anything else in the plant kingdom that the question of their morphological nature is a very puzzling one. Various opinions have been held. They have been interpreted as equivalent to sporangia, to lateral branches in the axils of leaves, to lateral branches without supporting leaves, to reduced branches resulting from dichotomy of the stem apex and gradually displaced, and to the bulblets of certain ferns and monocotyledons.

We owe the first accurate account of the development of the bulbils to Hegelmaier (4). He corrected the view previously held that they originate in the axils of preexisting leaves, and showed that they take the place of leaves in the phyllotactic spiral. He could find no means of distinguishing very young leaves and bulbils in longitudinal sections; and a view of the apex of the plant showed a difference only when the larger size indicated that a bulbil was beginning to develop.

Strasburger (10) gave a short description of the bulbils, differing from Hegelmaier's in some respects. In the following year, after further investigation (11), he confirmed the interpretation of Hegelmaier in every detail but one; he thought leaves and bulbils differ somewhat in their mode of development. By special manipulation of the stem tips, he selected for study those tips on which young bulbils were present. In these young bulbils, when viewed in longitudinal section, he thought he could detect a middle apical group of two cells in the dermatogen. This account does not contradict that of Hegelmaier, for Strasburger really found no difference between leaves and bulbils until the latter were distinguishable by their greater size. My observations on this feature agree with HEGELMAIER'S. I have not been able, in longitudinal sections, to distinguish a young bulbil from a young leaf until it is recognizable by the beginning of an apical meristem at its tip. STRASBURGER discussed the homology of the bulbil with considerable care, and after considering the possible explanations concluded that all the facts of development and histology are best combined in the supposition that the bulbil represents the survival of an original dichotomy ("eine einst dichotomischen Ursprung").

Much the same interpretation is given by CAMPBELL (1), who says the bulbils "are formed apparently in the axils of somewhat modified leaves. . . . . The axillary origin of the bulbils is only apparent; they are really, so far as can be determined, similar in origin to ordinary branches and formed without any relation to the leaves."

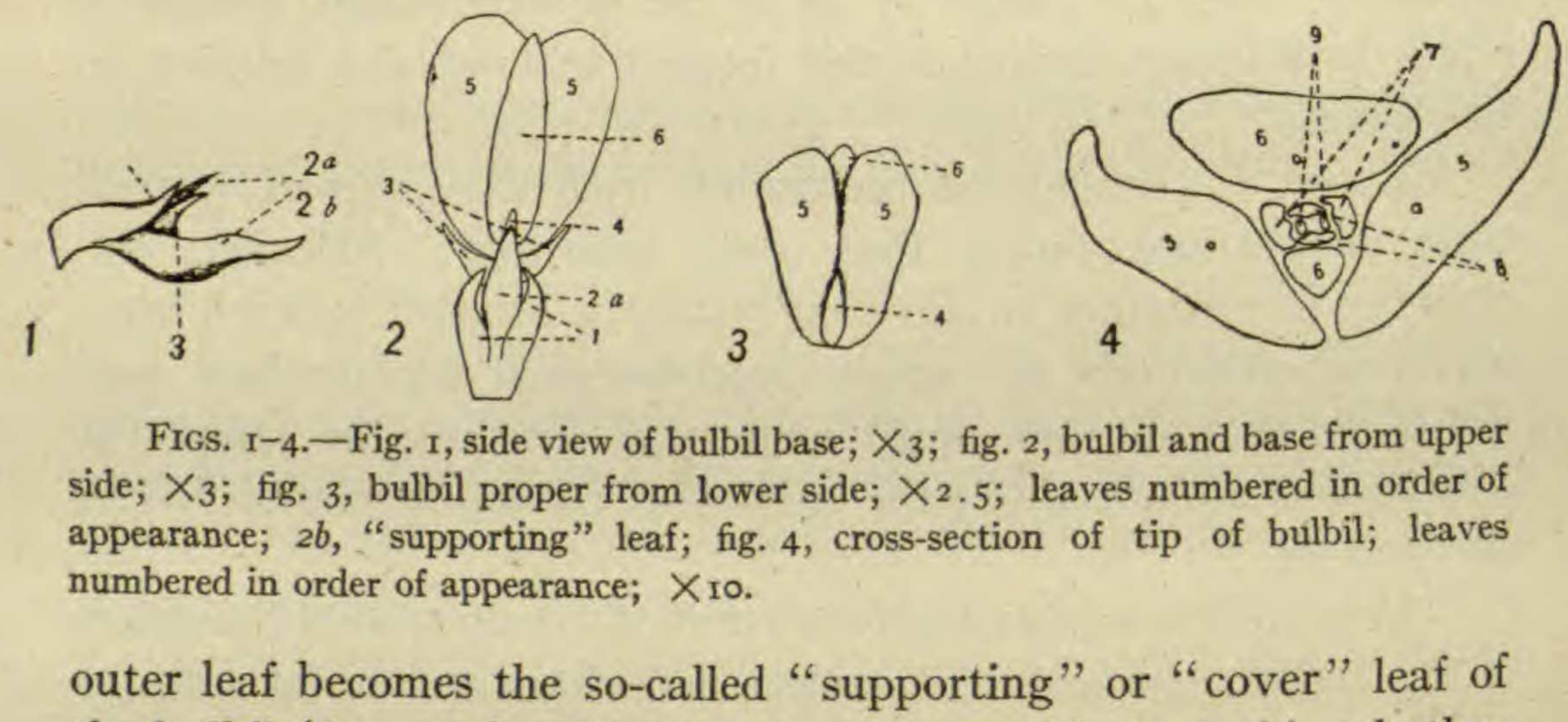
GOEBEL (3) considered the bulbils from the point of view of their adaptations rather than their homology. Although disclaiming any attempt to solve the latter, he brought forward some pertinent objections to current morphological explanations, and concluded that in their most important features the bulbils of *Lycopodium* are not unlike the bulblets of certain species of *Allium* and *Lilium*.

The present investigation is an attempt to help solve the problem of the bulbils by means of modern technique and serial sections which were not available to the earlier investigators. Only *L. lucidulum* has been studied, suitable material of other species not being obtainable. STRASBURGER, however, found that there is a great similarity in the bulbils of the six species which he compared, and it is probable therefore that the following account with only minor changes will hold good for all.

Description of bulbils

Two parts must be distinguished in the bulbil: the base which remains attached to the stem of the plant for many years and does not store up nutriment; and the distal part, or bulbil proper, which is heavily laden with starch grains, and which, when mature, separates from the base and readily gives rise to a new plant. Between the two parts is a narrow neck which breaks easily when the bulbil is ripe. The young bulbil has a small apical meristem at its tip, and as it grows produces decussate pairs of protuberances, which in their relation to the bulbil axis resemble foliar organs, but in maturity differ more or less from typical leaves. The base has three pairs of these and the bulbil proper about six.

From the bulbil primordium there arise, as described by HEGELMAIER from external appearances, first a pair of small leaves, laterally situated, but soon displaced toward the stem apex; then from the growing point between these a median pair, and soon after them another lateral pair, not displaced. The median pair, especially its abaxial member, is much the largest. This larger



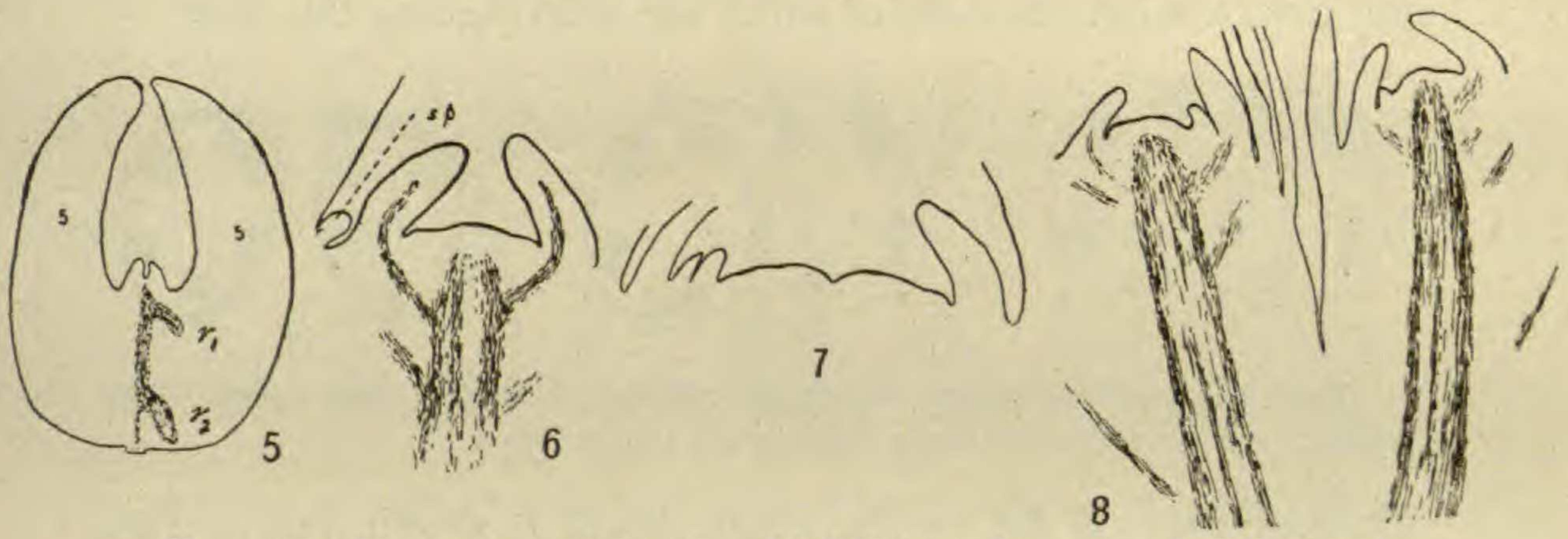
FIGS. 1–4.—Fig. 1, side view of bulbil base; ×3; fig. 2, bulbil and base from upper side; ×3; fig. 3, bulbil proper from lower side; ×2.5; leaves numbered in order of appearance; *2b*, "supporting" leaf; fig. 4, cross-section of tip of bulbil; leaves numbered in order of appearance; ×10.

outer leaf becomes the so-called "supporting" or "cover" leaf of the bulbil (figs. 1, 2). HEGELMAIER was unable to decide whether the further axis of the bulbil is the direct continuation of the primordium, and the supporting leaf therefore truly lateral, or whether the supporting leaf is the direct continuation of the primordium, and the bulbil proper therefore an outgrowth and truly lateral.

The first leaves of the bulbil proper are dorsal and ventral; the second pair, which are lateral in origin, gradually turn their upper edges outward and assume a dorsiventral relation; the third pair are median, long, and narrow (figs. 2, 3). These three pairs of leaves are fleshy, and the leaves of the second pair are also much modified in form. The remaining leaves of the bulbil are small and closely arranged about the growing point (fig. 4). The axis of the bulbil proper develops endogenously two small rootlets, which remain imbedded in the fleshy leaves until the germination of the bulbil, when they push out into the soil.

Anatomy of stem apex and bulbils

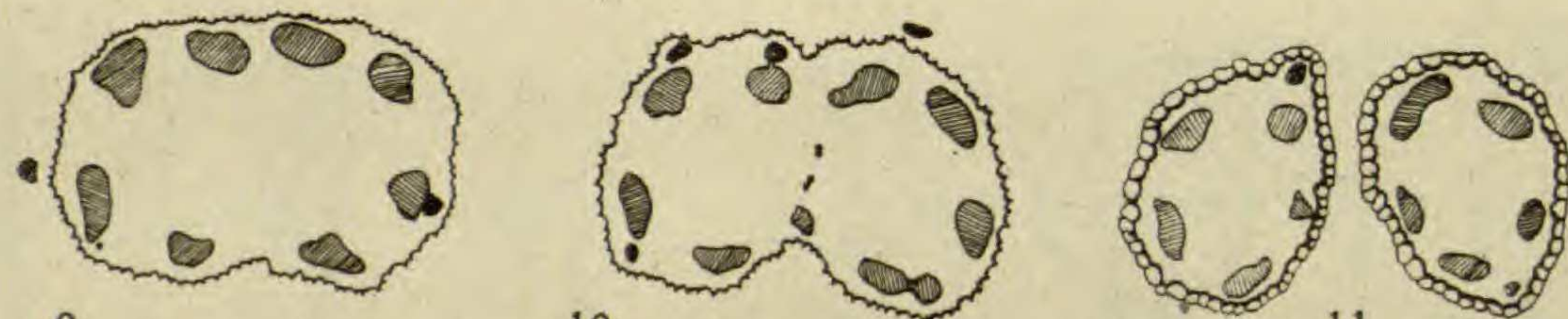
L. lucidulum has only one mode of branching, namely, by dichotomy of the apex. The growing point of the stem tip is broad and flat, or slightly raised in the center, and is surmounted and protected by the more rapidly lengthening leaves. In *Lycopodium* there seems to be a relation between the form of the growing point and the rate of growth. Slow growers, such as *L. lucidulum* and *L. Selago*, have the apex as described (fig. 6); while rapid



FIGS. 5–8.—Fig. 5, tangential section of bulbil: r_1 and r_2, roots; *s*, fleshy leaves; ×5; fig. 6, apex of stem: *sp*, young sporangium; ×38; fig. 7, beginning of dichotomy of stem in longitudinal section; ×38; fig. 8, more advanced stage of same; ×23.

growers, such as *L. clavatum*, *L. annotinum*, and *L. alopecuroides*, have the apex conoid in form and considerably in advance of the youngest leaves. Before the apex branches it becomes wider (there is considerable variation in the width of the growing point without any apparent relation to branching). The initial cells of the middle region now cease to grow and multiply, while those on either side continue their activity (fig. 7). Soon the two growing points, separated by a cleft, begin putting forth leaves on all sides (fig. 8). In longitudinal section it can be seen that the central cylinder has divided into equal strands, and the two apices are similar in all respects.

In cross-sections the equality of the two branches is just as apparent. Fig. 9 shows a section of the central cylinder below a dichotomy at a distance of about half a year's growth from the two tips. The central part of the cylinder is filled with immature tissue, which can be distinguished approximately into xylem and phloem areas. The shaded areas represent fully differentiated xylem made up on the outer side of protoxylem tracheids, and on the inner side of larger metaxylem elements. As differentiation proceeds in this species, the metaxylem portions meet in the middle of the cylinder and become more or less continuous, and the phloem occupies the indentations or bays between the xylem strands. Surrounding the cylinder there is a clearly marked endodermal sheath in the form of an irregular layer of cells, only the inner contiguous walls of which are thickened at this stage of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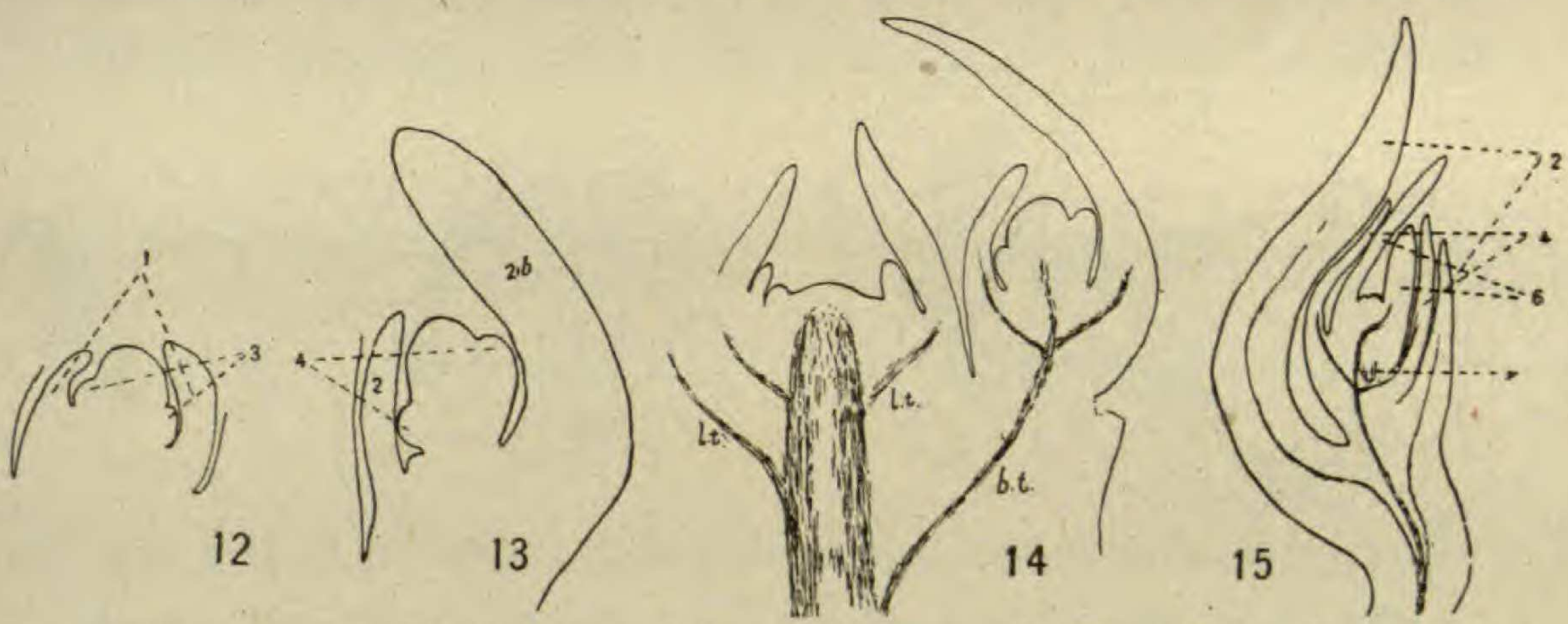
FIGS. 9–11.—Cross-sections of central cylinder of forking stem, xylem areas shaded; small cross-hatched areas indicate leaf traces; ×52.

development. In fig. 11 the whole sheath is shown, but in figs. 9 and 10 only the thickened cutinized walls are to be seen. As the dichotomy proceeds the xylem strands change position but little, the sheath from opposite sides pushing inward until the division into two similar cylinders is complete. In this mode of branching no branch gaps of any kind are formed in the sheath or xylem or phloem. The length of stem between figs. 9 and 10 was 0.65 mm.; and between figs. 10 and 11 it was 0.5 mm. It will be noted that there are eight xylem strands in fig. 9, and that in each of the two branches there are five. The new strands are not formed by a division of old ones, but arise *de novo* out of meristematic tissue. The beginnings of these strands may be seen in fig. 10.

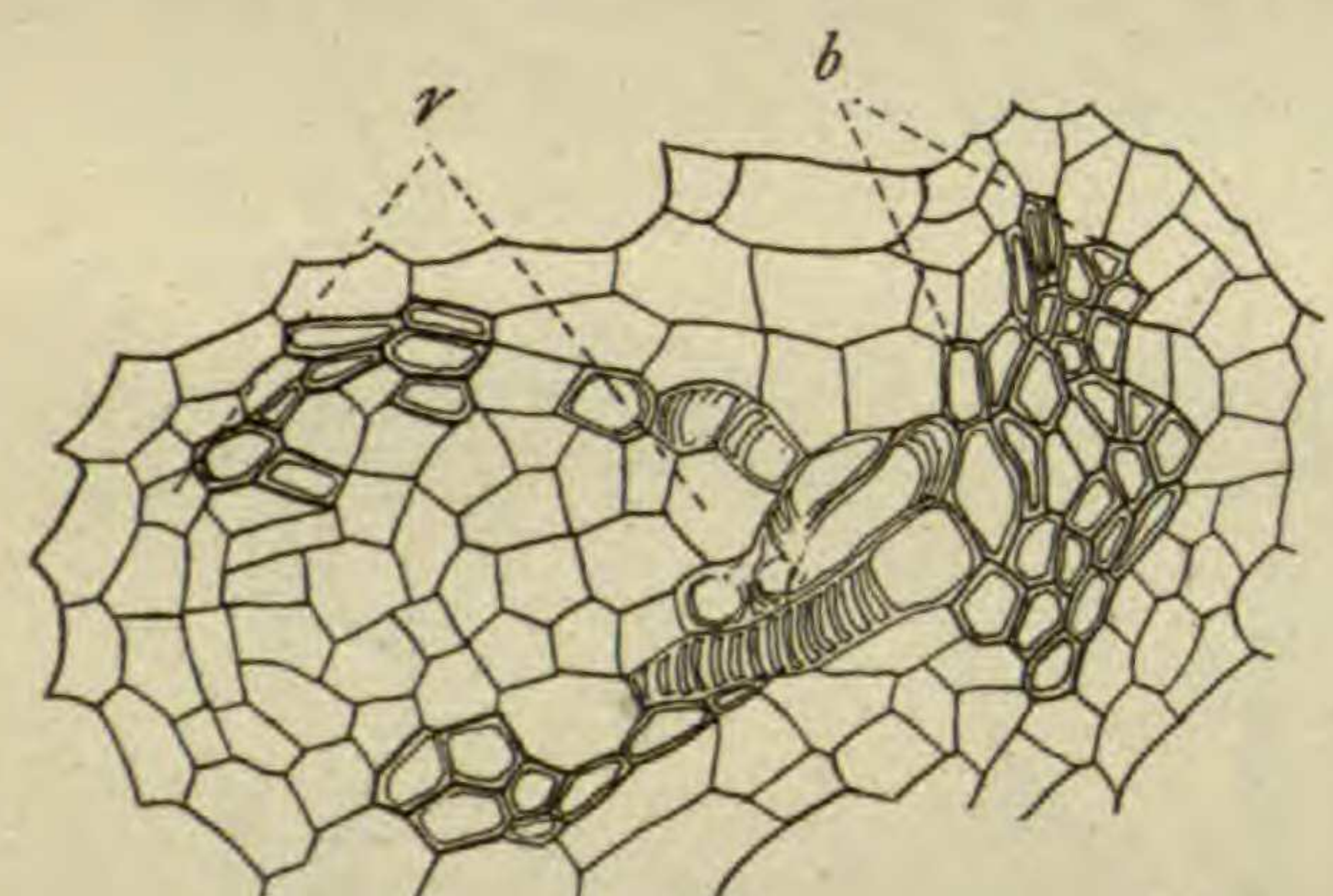
As shown by JONES (5), the protoxylem strands in *Lycopodium* are constant neither in number, shape, nor position. New strands may arise either by branching of preexisting strands, or from the meristem. I have also found that a strand when traced upward may gradually diminish in size and ultimately disappear. In the stem of which figs. 9, 10, and 11 are sections, the leaves were 9-ranked, and this ranking continues in both the branches, notwithstanding the reduction of protoxylem strands from nine to five. As shown many years ago by CRAMER (2) and HEGELMAIER (4), there is no relation between the number of orthostichies and the number of xylem strands. The leaf traces of *Lycopodium*, so far as they have been investigated, are mesarch in structure (7, 8). They are so in *L. lucidulum*. The trace separates very gradually



FIGS. 12–15.—Fig. 12, tangential section of young bulbil: *1*, first pair, *3*, third pair of lateral leaves; fig. 13, radial section of bulbil rather older than fig. 12; *2*, first pair of median leaves; *4*, first leaves of bulbil proper; ×38; fig. 14, longitudinal section of stem tip and young bulbil: *2b*, "supporting" leaf; *lt*, leaf trace; *bt*, bulbil trace; ×23; fig. 15, longitudinal section of older bulbil, leaves numbered in order of appearance; *r*, root tip; ×10.

from the protoxylem of the central cylinder, traverses the pericycle for a short distance, and then pierces the endodermis, after first pushing it out into the form of a little pocket (figs. 10, 11). The separation of the leaf trace causes no gap or disturbance in the central cylinder. Immediately above and below the point of exit the sheath closes. The leaf trace at first consists only of spiral tracheids, but later is surrounded by deeply staining cells, presumably phloem.

The vascular strand of the bulbil does not at all resemble that of a branch. It is a single strand, similar in every way to a leaf trace in its relation to the central cylinder and its passage through the cortex (fig 14). Not until it reaches the outer part of the cortex or the decurrent base of the bulbil does it exhibit any essential deviation from a leaf trace. Here it widens into a narrow tangential band of cells, and presently gives off laterally two small traces to the first leaves of the bulbil (fig. 12). The middle part continues and presently widens into a radial band whose outer portions become the traces of the second pair of leaves (figs. 13, 15), and so on until four pairs of traces have separated from it. These facts make it clear that the "supporting" leaf is undoubtedly a lateral organ. In the bulbil proper in connection with the appearance of



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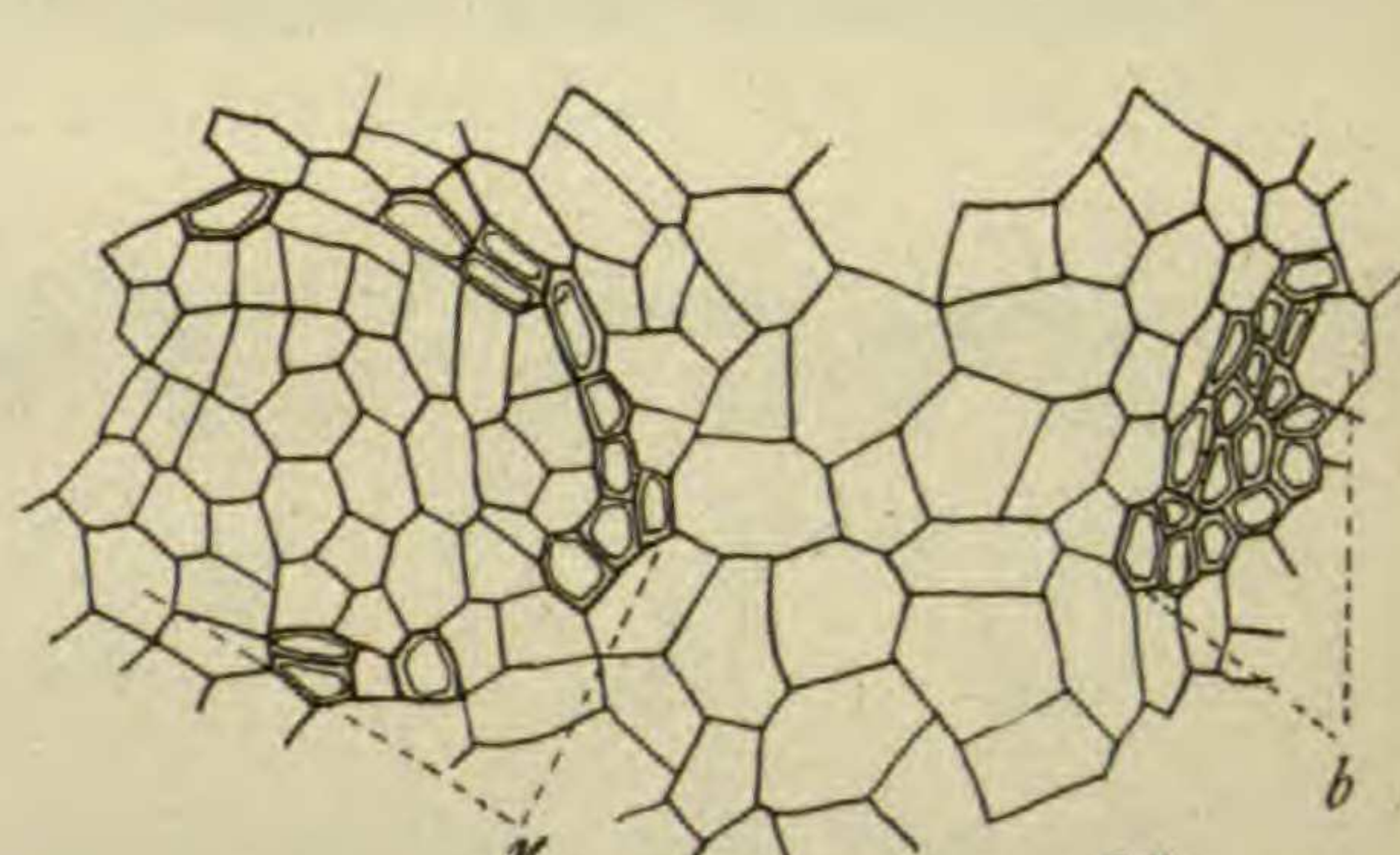

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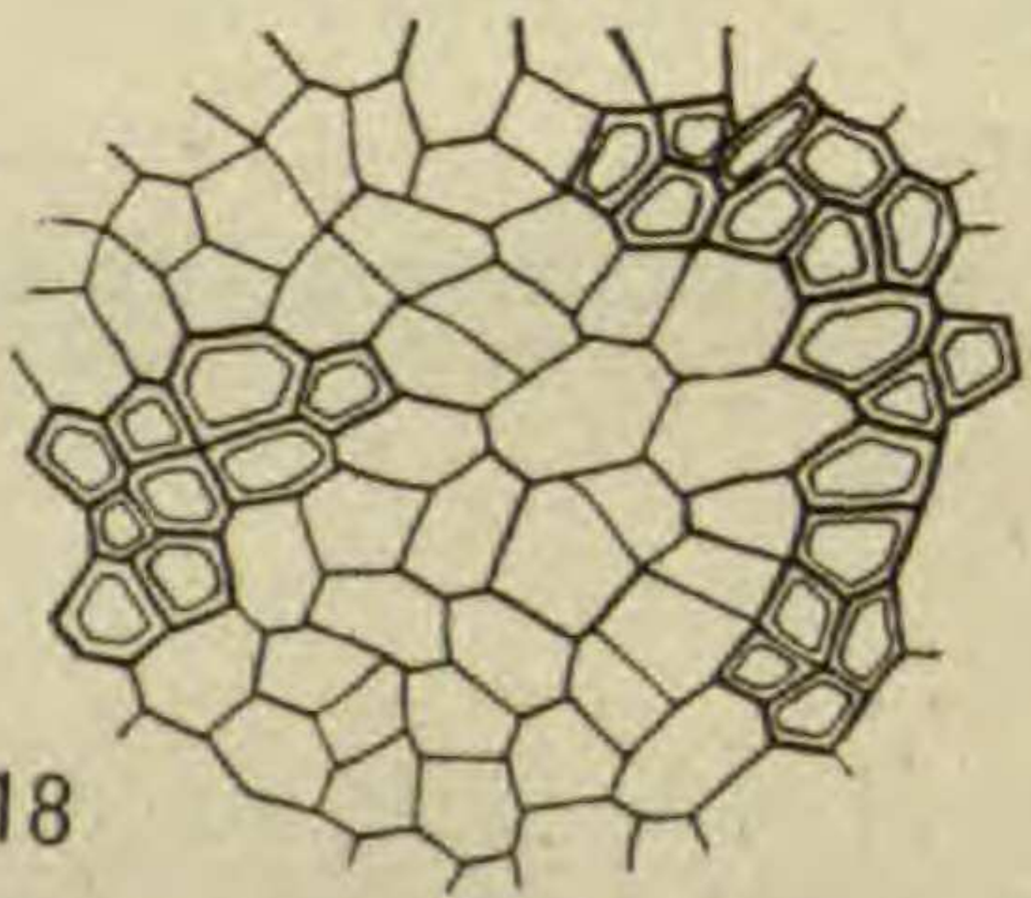
FIGS. 16, 17.—Fig. 16, cross-section of vascular bundle of bulbil where first root is given off; ×320; fig. 17, cross-section of bundles of bulbil and root below origin of root: *b*, bulbil bundle; *r*, root bundle; ×320.

the first root a much greater change occurs. In a young bulbil, even before the vascular tissues are differentiated, the tip of this root is outlined as a mass of deeply staining meristematic cells. A second and smaller root develops nearer the tip of the bulbil (fig. 5). Both of these roots connect with the vascular strand of the bulbil on its adaxial side and, turning downward and laterally, remain imbedded in the parenchyma and separated from it by slime and crowded tissue.

Fig. 16 shows the junction of the root cylinder and the axis of the bulbil, and fig. 17 is a section below this. In both of these it can be seen that the bulbil axis is still essentially a leaf trace, but above the junction of root and axis all resemblance to a leaf trace is lost. The arrangement of the tracheids is somewhat like that of a root, but much more irregular. Sometimes it is distinctly diarch (fig. 18), more commonly it is horseshoe-shaped, and sometimes almost a complete ring, often with a few tracheids in the center (fig. 19). Surrounding the whole xylem area there are one or two irregular layers of cells, probably a pericycle, and outside these an indistinct sheath (this feature is shown only in fig. 16). This irregular arrangement continues until the fifth and sixth pairs of leaf traces are given off and also the second root. Above that the tissues are still meristematic in the ripe bulbil. The bulbil, like the leaves, is well supplied with stomata, and photosynthesis proceeds actively. The cause of the accumulation of starch in the bulbil proper and not in the base seems to lie in the absence of phloem in the neck of the bulbil. Here the cells are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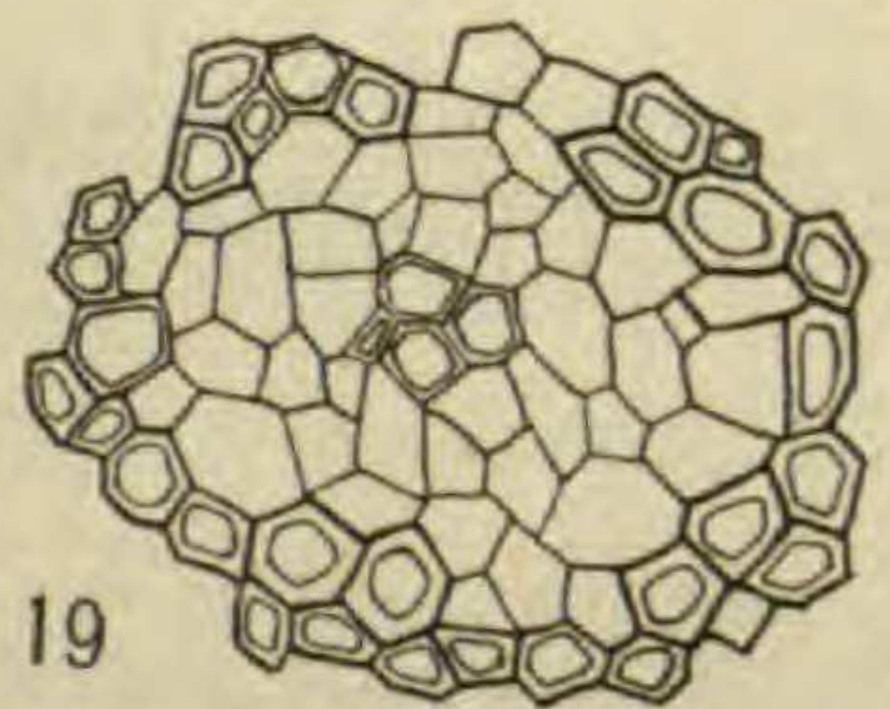
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FIGS. 18, 19.—Cross-sections of bundle above origin of first root; ×320

entirely wanting which elsewhere in the bulbil surround the xylem strand, and which I have regarded as phloem, although the sieve plates have not been identified. The detachment of the bulbil proper is brought about by a disorganization, apparently a gelatinization, of the walls of the xylem cells in the neck.

When a ripe bulbil is kept on damp soil it soon germinates. Both roots penetrate the soil and branch repeatedly, and from the tip of the bulbil a slender stem is put forth. On this stem the new leaves, although scattered, are from the first arranged spirally. JONES (5) traced the development of the vascular cylinder in the young plants growing from bulbils of *L. Selago* and *L. serratum*, and concluded that the original simple bundle becomes successively diarch, triarch, etc., by division of the xylem. My observations on *L. lucidulum* do not agree with this conclusion. I find, in agreement with JONES, that the young stem at its attachment with the bulbil is diarch, having two masses of xylem with one phloem mass between, but followed up for nearly an inch, it never becomes triarch. The xylem becomes horseshoe- or ring-shaped, much as in figs. 17 and 19, changing constantly, and there is no hint as to how the multiprotoxylic condition of the adult stem arises. The irregularity and continual change in the xylem of these young stems are comparable to the conditions described by Miss WIGGLESWORTH (12) in young *Lycopodium* sporophytes growing from gametophytes.

HEGELMAIER'S statement that the bulbils replace leaves in the phyllotaxy is confirmed by an examination of the relation of bulbil and leaf traces. On following these back in cross-sections to their junctions with the xylem strands of the central cylinder, the bulbil traces are found in close succession. In many cases they represent successive leaves; in other cases a leaf trace stands between two bulbil traces; but in every case examined the bulbil traces all fell within a single leaf spiral.

Conclusions

1. The bulbil is not the homologue of a branch, since it has a simple vascular strand with mesarch concentric arrangement; whereas branches have a vascular system with complex exarch radial arrangement. Furthermore, it is not a reduced dichotomy or the equivalent of the bulblets of *Lilium* or *Allium*.

2. It is not the homologue of a sporangium, for the reason, among others, that it receives a prominent vascular strand, a feature which is lacking in the sporangia of all Lycopodiales.

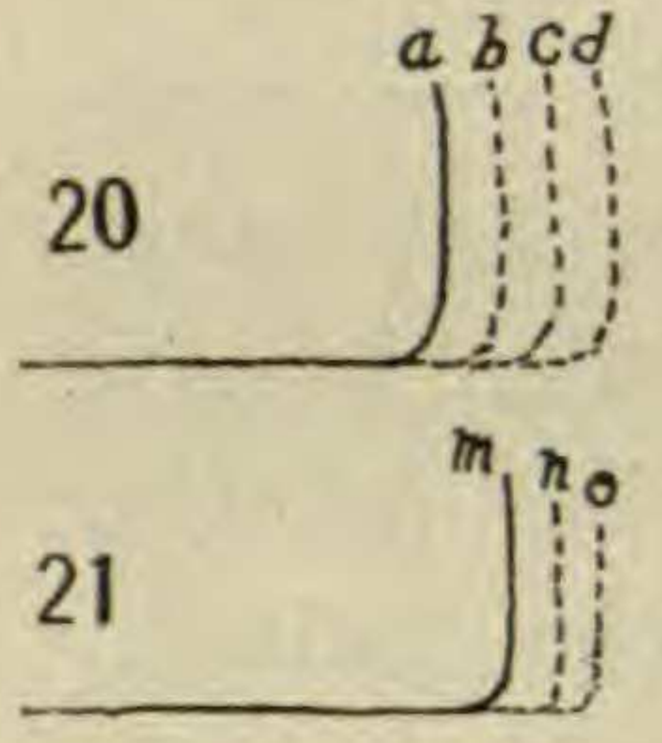
3. It is a transformed leaf, retaining the position, dorsiventrality, and in great measure the vascular strand of a leaf. It may perhaps be homologized with the bulblet of a fern produced so early in the history of the leaf bearing it that the latter exhibits the leaf character only while inside the cortex of the stem.

Further observations on the bulbils and habits of L. lucidulum

In the literature of the subject, I do not find any recognition of the periodicity of the bulbils. In *L. lucidulum* they are quite as responsive to season as the sporangia. The latter begin to form in autumn in preparation for the next year, and continue to form when growth is renewed in the spring. Then, beginning some time in June, for more than two months the apex develops a succession of sterile leaves, in the midst of which two to four bulbils may make their appearance, always near each other and on one side of the stem, on that side of a branch which is farthest from the other branch. The primordia of the bulbils increase rapidly in size, pointing vertically upward at first like the young leaves, and then gradually becoming horizontal. They reach maturity in September. Since the bulbils are formed but once a year and their bases are persistent, it follows that they furnish a means of measuring the annual increment of growth in length. There are other but less accurate means of estimating a year's growth, by the old sporangia whose walls do not completely disappear for several years, and also by a difference in the length of sporophylls and sterile leaves. The annual growth in plants bearing bulbils can be determined quite easily for the last ten or twelve years, and in some cases even for twenty years. It is surprisingly small, averaging about one inch.

The possibility of determining the age of any given part of the stem leads to some interesting observations. For instance, it can be shown that the metaxylem matures very slowly, not being fully differentiated until well into the second year. The cortical zones, characteristic of most *Lycopodium* stems, are not differentiated until the third year. It is also possible to calculate the frequency of dichotomy of the stems. The commonest interval between successive dichotomies is four years, but intervals of two, three, five, and six years also occur frequently. I have never found a stem branching in two consecutive years.

Like several other species, *L. lucidulum* has the old stem horizontal and rooted, and the young and greener stem upright. By means of the bulbil bases it is clearly shown that the upright part is six or seven years old. Observations from year to year show that the plants, although adding annually one inch to their length, maintain the same average height. Each year a part of the erect stem, equaling a year's growth, becomes curved and finally horizontal. Fig. 20 represents the situation in four successive years. Decapitated plants are not killed, but, having no means of renewing the apical meristem, they cease to grow or divide. Fig. 21 represents such a decapitated stem in three successive years, the upright portion shortened each year by the equivalent of a year's growth. Whether such a stem will finally become altogether horizontal, I do not yet know, nor have I yet been able to discover the cause of the regular change in direction from upright to horizontal. Observations in the field seem to show that root contraction is not the determining cause (stem tips decapitated in 1915 were found to be dead in the summer of 1919).



FIGS. 20, 21.—Fig. 20, diagram representing position of plant four successive years; fig. 21, diagram representing position of decapitated plant three successive years.

Some interesting data can also be gathered in regard to the behavior of the roots. These, as in many other species of *Lycopodium* whose tips are erect or sloping (6, 7, 9), do not pass straight out through the cortex, but turn downward. In *L. Selago* these roots "arising at the apex pass obliquely, then directly down through the middle cortex and only appear at the outside beneath the soil" (6). The same description is true of *L. lucidulum*, except that the roots do not originate at the apex, but begin at some distance behind the apex; how far behind is not clear. They rarely form within the first year's growth. Apparently they increase in number in the older parts of the erect stem; but as small roots might easily be overlooked in freehand sections, I am not quite certain that new roots may form in parts of the stem as old as five or six years. These roots, in the upright part of the stem, remain small, usually less than 6 mm. in length. As they do not emerge to the outside until in a part of the stem at least six or seven years old, it is evident that they must be in a dormant or slowly growing condition within the cortex for as many as two to five years. The downward growth in the cortex is not very great, the distance between the junction with the central cylinder and the point of emergence to the outside varying from 6 to 15 mm. Reaching the soil, they grow rapidly, quickly reaching a length of several inches.

Summary

1. The origin and vascular bundles of branches, leaves, and bulbils are described, and the conclusion is reached that the bulbil is comparable to a leaf rather than to a branch.

2. The accumulation of starch in the bulbil proper is ascribed to the absence of phloem in the narrow neck joining it to the base, and the detachment of the ripe bulbil to the disorganization of the xylem walls in this region.

3. The rate of growth is estimated from the persistent bases of the bulbils, and observations are made on the habits of the plant.

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